DOI: 10.1002/chem.200701345

Recognition Properties of Donor- and Acceptor-Modified Biphenyl-DNA

Alain Zahn and Christian J. Leumann*[a]

Abstract: The recognition properties of DNA duplexes containing single or triple incorporations of eight different donor-modified (OMe, NH_2) and acceptor-modified (NO_2) biphenyl residues as base replacements in opposite positions were probed by UV-melting and by CD and fluorescence spectroscopy. We found a remarkable dependence of duplex stability on the natures of the substituents (donor vs. acceptor). The stabilities of duplexes with one biphenyl pair increase in the order donor/donor < acceptor/donor < acceptor/acceptor substitution. The most stable biphenyl pairs stabilize duplexes by up to 6°C in T_m. In duplexes with three consecutive biphenyl pairs the stability increases in the inverse order (acceptor/acceptor < donor/acceptor < donor/donor) with increases in T_m, relative to an unmodified duplex, of up to 10°C. A thermodynamic analysis, combined with theoretical calculations of the physical properties of the biphenyl substituents, suggests that in duplexes with single biphenyl pairs the affinity is dominated by electrostatic forces between the biphenyl/nearest neighbor natural base pairs, whereas in the triple-modified duplexes the increase in thermal stability is predominantly determined by hydrophobic interactions of the biphenyl residues with each other. Oligonucleotides containing amino biphenyl residues are fluorescent. Their fluorescence is largely quenched when they are paired with themselves or with nitrobiphenyl-containing duplex partners.

Keywords: biaryls · DNA recognition · hydrophobic bases · oligonucleotides · pi interactions

Introduction

The contribution of stacking of the natural bases to DNA duplex stability has been a matter of debate since the discovery of the double helix. The importance of stacking has recently been highlighted in the context of oligonucleotide duplexes containing nonpolar nucleobase substitutes as dangling ends.[1,2] Factors such as hydrophobicity (log*P*), polarizability, dipole moment, surface area, and stacking area have been discussed as contributors to the observed enhanced thermodynamic stability.[2–4] In another context, shape mimics of complementary natural bases that were devoid of the potential to form hydrogen bonds have been investigated as probes for DNA-processing enzymes.[5–11] Although such isosteres destabilize DNA duplexes, they can code for each other with high precision in polymerase-mediated DNA replication. These findings triggered an intense search for novel, aromatic base substitutes that are orthogonal to the natural base pairs in their recognition properties and that could potentially be exploited for the extension of the genetic alphabet.[12–22]

In a non-biological context, DNA is becoming increasingly important as a scaffold for self-assembling nanometer-scale molecular entities.[23,24] In addition to this, the mechanism of charge transport through the base-stack of DNA has been extensively investigated in the past,[25,26] and is beginning to be applied for nucleic acid sensing.[26,27] In addition, the replacement of natural base pairs with metalated base pairs offers new perspectives in applications of DNA as a molecular wire or as a spatially addressable magnetic storage device on the nanometer scale.[28,29]

In our research directed towards the exploitation of interstrand aromatic interactions for producing novel and functional DNA duplex architectures we recently found that up to seven biphenyl *C*-nucleoside pairs can be accommodated in the center of a helix without loss of duplex stability.[30,31] From molecular modeling we proposed a zipper-like recognition model and assumed that the acquired stability is based on interstrand aromatic interactions between the biphenyl residues. This zipper model is now supported by a

[a] Dipl.-Chem. A. Zahn, Prof. C. J. Leumann
Department of Chemistry and Biochemistry
University of Bern
Freiestrasse 3, 3012 Bern (Switzerland)
Fax: (+41) 31-631-3422
E-mail: leumann@ioc.unibe.ch

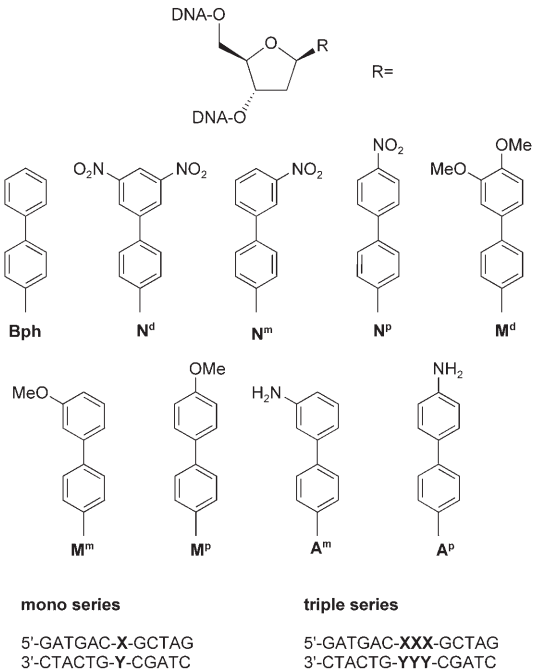
Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

recent NMR structure of a biphenyl-modified DNA decamer duplex.[32]

This zipper recognition motif leaves ample room for modification of the biphenyl periphery in order to modulate their functional and recognition properties. To investigate the influence of the π electron density of the biphenyl residues on duplex stability we prepared a series of biphenyl *C*-nucleosides with electron-donating and electron-withdrawing groups in the distal (remote) phenyl ring. Here we report on the recognition properties of duplexes containing eight differently substituted biphenyl pairs.

Results

The synthesis of the monomeric *C*-nucleoside building blocks and the corresponding oligonucleotides has already been described.[33] For determining the recognition properties, two standard sequence contexts were chosen (Scheme 1). One sequence (mono series) involved the single



Scheme 1. Chemical structures of the biphenyl-*C*-nucleosides, together with the corresponding investigated oligodeoxynucleotide duplexes.

incorporation of a biphenyl pair, while the other (triple series) was based on the inclusion of three contiguous biphenyl pairs in the center of the duplex. Sequences were designed to contain only one type of substituted biphenyl-*C*-nucleoside per strand (no mixed biphenyls in the same strand). As the sequences were not self-complementary, all permutational arrangements of donor- and acceptor-substituted biphenyls were possible and no interference from hairpin formation had to be allowed for.

Molecular properties of the substituted biphenyls were calculated by ab initio or semiempirical methods. Table 1 shows calculated values of the ionization energies, dipole moments, isotropic polarizabilities, and partition coefficients between water and octanol (log*P*). Additional UV and fluorescence spectroscopic data are given in Figure S1 (Supporting Information). For comparison these calculations were also performed with the natural purine bases adenine and guanine.

Table 1. Structural, physical, and electronic properties of substituted biphenyl compounds (free aromatic units) and of the two natural bases adenine (Ade) and guanine (Gua) for comparison. Gaussian 03 was used for the RHF calculations with 6-31G(d,p) as the basis set.

	IE[a] [eV]	μ_0[b] [Debye]	α[c] [Bohr^3]	log*P*[d]
N^d	9.3	5.8	141	3.6
N^m	8.8	5.3	127	3.7
N^p	8.9	5.8	129	3.7
Bph	8.2	0.0	112	3.7
M^m	8.1	1.3	128	3.8
M^p	7.7	1.4	129	3.8
A^m	7.8	1.5	120	2.8
M^d	7.6	0.5	144	3.4
A^p	7.4	1.7	122	2.8
Gua	8.1	6.8	–	–
Ade	8.4	2.5	–	–

[a] First ionization energy based on Koopman's theorem (IE = $-E$(HOMO)). [b] Dipole moment. [c] Isotropic polarizability. [d] Partition coefficient between water and octanol from the molinspiration semiempirical routine (http://www.molinspiration.com).

The calculated ionization energies of the two natural bases guanine and adenine are in very good agreement with experimental values (8.24 and 8.44 eV, respectively) determined by gas-phase photoelectron spectroscopy.[34] This shows that Koopman's theorem (IE = $-E$(HOMO)) is a simple but suitable method for the calculation of the first vertical ionization energy. The donor-substituted biphenyls show lower ionization potentials than the natural purine bases, whereas the potentials of biphenyls bearing electron-withdrawing groups are higher. The ionization potential of unmodified biphenyl is in the same range as those of the natural purines.

All nitro compounds show very high dipole moments in relation to the other substituted biphenyls. Biphenyl itself does not possess a permanent dipole moment for symmetry reasons. It is generally known that RHF calculations overestimate the dipole moments of the natural bases. Comparison with more accurate calculations of the dipole moments at the MP2/aug-cc-pVDZ level, however, shows close agreement of the results (μ_0 = 6.55 and 2.56 debye for guanine and adenine, respectively).[35] The isotropic polarizability is correlated to the size of the molecule and seems not to depend on the electronic properties of the substituents. The log*P* values are in the same range, with the exception of those of the two aminobiphenyl compounds, which show lower values. This can be explained by the hydrogen bond donor and acceptor properties of the amino group.

Mono series: To determine thermal stabilities we recorded UV melting curves for all possible duplexes under standard conditions. A representative subset of the data is graphically reproduced in Figure 1. The duplex with no biphenyl pair

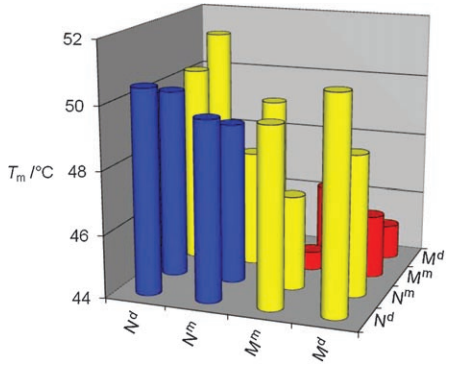


Figure 1. Graphical representation of the T_m values determined from UV melting curves of donor/donor, mixed, and acceptor/acceptor biphenyl duplexes of the mono series (see Scheme 1). Conditions: see Experimental Section.

(deletion mutant, $T_m = 45\,°C$) served as a standard. The T_m data are summarized in Table S2 (Supporting Information).

From the data it is evident that none of the biphenyl pairs destabilizes the parent duplex. On the contrary, most of the biphenyl pairs increase duplex stability by up to 6 °C, which is more than the stabilization by an additional AT base pair, for which a T_m of 47.9 °C was determined. It is immediately evident that the acceptor-modified biphenyls stabilize the duplex most (4–6 °C). The mixed donor–acceptor pairs also stabilize the duplex, although showing greater T_m diversity (2–6 °C). Interestingly, donor-modified biphenyls do not significantly stabilize the duplex. Here the T_m increases are in the 0–2 °C range relative to the deletion mutant.

To analyze the effect of the substitution patterns on duplex stability we compared the T_m data for *meta*- and *para*-substituted biphenyls (Figure 2, Table S3, Supporting Information). We found no significant differences in T_m arising from the position of substitution and therefore conclude that steric effects play only a minor role in the recognition properties of the mono series.

Bulge position: To investigate the interaction of the biphenyls with the natural bases only, all eight substituted biphenyls were tested in their ability to insert into the DNA base stack by intercalation (Table 2).

Table 2. T_m data from UV melting curves: $c = 1.2\,\mu M$ in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0. Estimated error in $T_m = \pm 0.5\,°C$.

	5′-GATGAC-**X**-GCTAG-3′ 3′-CTACTG-CGATC-5′		
X	T_m [°C]	X	T_m [°C]
N^d	51.1	M^d	43.3
N^m	51.0	N^p	49.9
M^m	48.7	M^p	45.3
A^m	44.7	A^p	41.1

The T_m data clearly demonstrate that oligonucleotides with acceptor-substituted biphenyls also form more stable duplexes in bulge positions (T_m up to +6 °C relative to the unmodified duplex). Again, the donor-substituted (OMe) biphenyls show a more heterogeneous picture ($T_m = -4$ to +4 °C). Substitution at the *meta* position seems to be slightly favored over *para* substitution, especially in the case of donor modification. This is somewhat at variance with the observation made in the mono series (see above), where almost no influence of the substitution pattern was found.

Triple series: As in the case of the mono series, the T_m data (Figure 3, Table S4, Supporting Information) were also measured for the triple series. As expected, all triple-modified duplexes are more stable than the mono-modified ones. However, substantial differences in T_m as a function of the nature of the biphenyl substituent are also observed in these cases. Interestingly, addition of any acceptor biphenyl pair(s) to the first one did not lead to any significant increase in thermal duplex stability. The opposite is observed

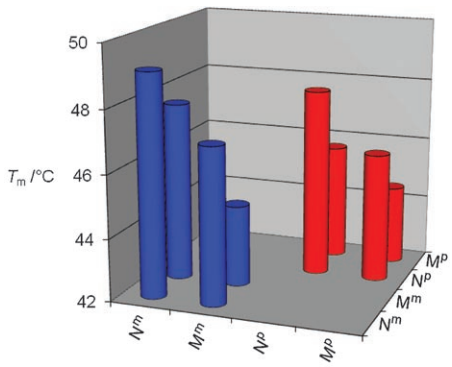


Figure 2. Graphical representation of the T_m values from UV melting curves of *para*- and *meta*-substituted biphenyl duplexes of the mono series (see Scheme 1). Conditions: see Experimental Section.

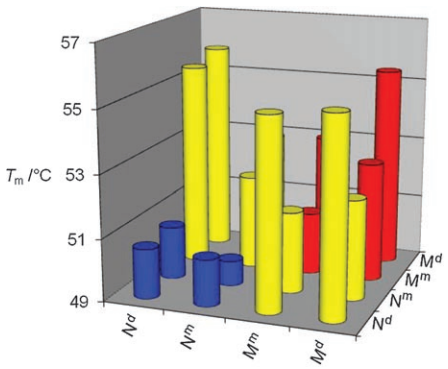


Figure 3. Graphical representation of the T_m values from UV melting curves of donor/donor, mixed, and acceptor/acceptor biphenyl duplexes of the triple series (see Scheme 1). Conditions: see Experimental Section.

in the case of donor substitution, where the additional interactions lead to higher stability, with the dimethoxy-substituted system being the most stable. Relative to the deletion mutant, the T_m increase for the dimethoxy-substituted duplex amounts to roughly 10 °C (3.3 °C per pair). Again, the T_m values for the mixed donor/acceptor pairs are located in-between, with the dinitro/donor arrangements being more stable (almost of equal stability to the dimethoxy system) than the mononitro/donor arrangements.

Mono versus triple series: Two different interactions of the biphenyl residues have to be taken into account when analyzing these results: firstly, the interaction of the biphenyl residues with their neighboring natural base pairs, and secondly, the interaction of the biphenyls with each other. If it is assumed that the zipper-like interstrand intercalation model, found by NMR for the single series,[32] also applies to the triple series, the interactions with the neighboring natural base pairs clearly dominate in the mono series (two contacts with the neighboring natural base pairs and one interbiphenyl contact), while in the triple series the interbiphenyl contacts dominate (two contacts with the neighboring natural base pair and five interbiphenyl contacts). To determine the contribution to the thermal stability of the interbiphenyl interactions exclusively we plotted the ΔT_m values between the triple and the mono series. As can be seen from Figure 4 it clearly emerges that acceptor biphenyl interactions do not contribute much to the duplex stability, while donor biphenyl interactions do so significantly. Again, the T_m values for the donor/acceptor arrangements are in-between.

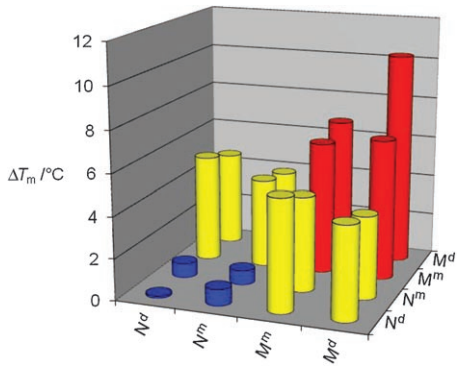


Figure 4. Graphical representation of the ΔT_m values (triple−mono series, Scheme 1) from UV melting curves. Conditions: see Experimental Section.

As before, the influence of the biphenyl substitution patterns on duplex stability was investigated. We found a strong difference between *meta*- and *para*-substituted biphenyls (Figure 5, Table S5, Supporting Information). Substitution in the *para* position seems to be more favorable in all cases, regardless of the electronic character (donor or acceptor) of the substituent.

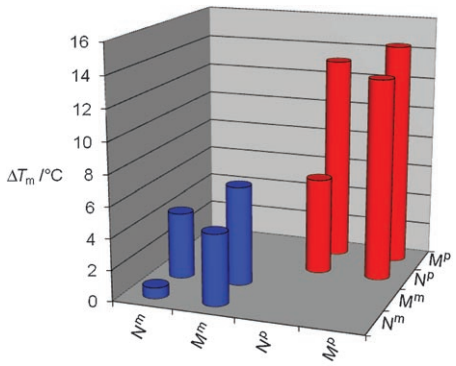


Figure 5. Influence of the substitution pattern on ΔT_m (triple−mono series, Scheme 1). Conditions: see Experimental Section.

Thermodynamic data for duplex formation: To determine the origins of the differences in thermal stability, the free energies of duplex formation ($\Delta G^{298\,K}$) were derived from plots of $1/T_m$ against $\ln c$ (Figure S1, Supporting Information) for duplexes containing one or three dinitro- or dimethoxybiphenyl pairs (Table 3). As expected, we found higher thermodynamic stability of the triple-modified dimethoxy duplex over its mono-modified counterpart, by almost 6 kcal mol^{-1}. Interestingly, the higher thermodynamic stability is largely due to a more favorable pairing enthalpy term (ΔH). In the case of the dinitro series a slight reduction of the ΔH term between the single- and the triple-modified duplexes was found.

Table 3. T_m data from UV melting curve analysis and thermodynamic data from van't Hoff analysis. $c = 1.2$ μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0. Estimated error in $T_m = \pm 0.5$ °C.

X–Y	n	5′-GATGAC-(**X**)$_n$-GCTAG-3′ 3′-CTACTG-(**Y**)$_n$-CGATC-5′ $\Delta G^{298\,K}$ [cal K^{-1} mol^{-1}]	ΔH [kcal mol^{-1}]	ΔS [cal K^{-1} mol^{-1}]
N^d–N^d	1	−15.1	−78.1	−211.5
N^d–N^d	3	−15.0	−75.9	−204.6
M^d–M^d	1	−14.0	−79.3	−219.1
M^d–M^d	3	−19.7	−114.1	−316.9

CD spectroscopy: While the overall structure in the mono series is known from NMR,[32] corresponding information for duplexes containing more than one biphenyl pair is not yet available. To obtain some preliminary data on the influence of multiple substitutions on the helix parameters, CD spectra of selected duplexes in the triple series were measured (Figure 6). Each CD spectrum shows the typical shape of a right-handed helix of the B type. The red shift of the ellipticity maxima of the OMe-substituted biphenyl duplexes correlates with their red-shifted UV absorption maxima and is consistent with these units being in the chiral environment of a stacked structure (induced CD). CD spectra at different temperatures reflect cooperative structural transitions

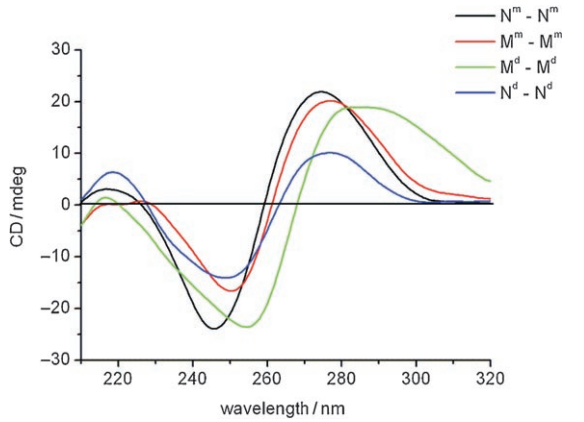


Figure 6. CD spectra of selected duplexes of the triple series: $c = 3.6$ μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, $T = 20$ °C.

around the expected melting temperatures (Figure S2, Supporting Information).

Fluorescence measurements with amino-substituted biphenyls: In contrast to the nitro- and the methoxy-substituted biphenyls, the amino-substituted ones are fluorescent. We therefore investigated the fluorescent properties of the *para*-aminobiphenyl residues in duplexes of the triple series both with themselves and with the nitrobiphenyls that act as fluorescence quenchers in the opposite positions. The *para*-substituted aminobiphenyl was given preference, due to its more red-shifted excitation and emission wavelengths.

When the *para*-aminobiphenyls were placed opposite dinitrobiphenyls, strong decreases in fluorescence intensity were observed (Figure 7). Upon melting, the fluorescence intensity is increased twofold to the level of that of the single strand alone.

The aminobiphenyl unit also shows self-quenching. Paired with itself in a duplex, fluorescence is reduced twofold and upon melting is restored to an intensity that corresponds to the sum of the single-strand fluorescence. No signs of excimer formation were observed.

Both quenching results show that the biphenyl moieties must be in close contact. The control shows that there is no significant influence of the temperature on single-strand fluorescence. Comparison of the single strands containing one and three A^p modifications shows that intrastrand quenching of the biphenyls in the single stand is not very pronounced. The triple-modified strand therefore shows almost three times enhanced fluorescence intensity (Figure S3, Supplementary Information).

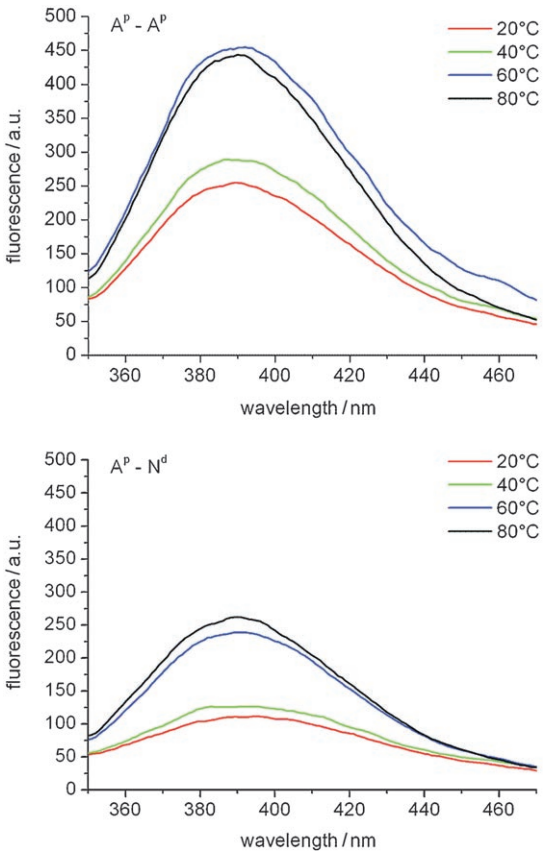


Figure 7. Fluorescence emission spectra, at different temperatures, of a duplex containing three *p*-aminobiphenyl nucleotide (A^p) units in both strands (top) and of a mixed duplex with the A^p–N^d combination (bottom): ${}^{ex}\lambda_{max} = 278$ nm; slit: 5 (ex.)/10 (em.); $c = 1.2$ μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0.

Discussion

Biphenyl–natural base pair interactions in the mono series: The presented structure/affinity relationship for duplexes containing single biphenyl pairs showed a strong dependence of T_m on the nature of the substituents (donor vs. acceptor). The stabilities increase in the order donor/donor < donor/acceptor < acceptor/acceptor pairs. A recent NMR structure of a decamer duplex containing a dinitrobiphenyl/dimethoxybiphenyl pair gave a detailed picture of the relative arrangement of the biphenyls in the duplex. Tight intrastrand face-to-face stacking of the proximal biphenyl ring on an adjacent 3′-guanine base was observed, determining the order by which the biphenyls recognize each other through interstrand stacking contacts.[32] If it is assumed that the interactions with the adjacent natural base pairs are energetically dominant, the preference of acceptor biphenyls can be explained by more favorable interaction of these electron-poor aromatic compounds with the adjacent electron-rich natural guanine bases in the 3′-direction (quadrupolar interactions, electrostatic). Further evidence comes from the observation that acceptor-substituted biphenyls are also found to be more stabilizing than donor-substituted biphenyls in bulge positions between two CG base pairs. Quadrupolar effects have been demonstrated to be important in different model systems, especially if aromatic units are oriented in a face-to-face orientation.[36–38] In addition, our calculations

show that all nitrobiphenyls possess high permanent dipole moments and could therefore interact with the neighboring natural bases through favorable induced dipolar interactions. This effect would be expected to be particularly strong with an adjacent natural guanine base because it possesses the highest polarizability of all four natural bases.[39]

Another driving force for stability could be the dispersive forces (momentary dipole–induced dipole interactions) between two aromatic entities. These are known to depend on the polarizabilities and on the extent of overlap of the interacting partners. Given the fact that our calculations did not show any significant differences in polarizability α between structurally matching pairs of nitro- and methoxy-substituted biphenyls (Table 1) we conclude that these effects are of minor importance and do not explain the observed stability differences.

Previous studies with duplexes containing aromatic units as dangling ends show that inductive and electrostatic effects play a minor role relative to solvation in helix stabilization.[2] The structural context here is different from the dangling end situation insofar as the aromatic units are completely integrated in the DNA base stack and have no surface with direct exposure to the solvent. Unless there are no major structural and energetic differences in the single strands this would imply that solvation was a less important criterion in interpretation of the observed relative stabilities. Together with the arguments above, we therefore conclude that electrostatic contributions are energetically relevant in the mono series. We explicitly mention here that the situation is different in the case of the triple series (see below).

Biphenyl–biphenyl interactions in the triple series: Surprisingly, the investigation of the triple-modified duplexes showed thermal stabilities increasing in the order acceptor/acceptor < donor/acceptor < donor/donor. This is the opposite of what was observed in the mono series and is also counterintuitive, as electrostatic repulsion would be supposed to destabilize a tight arrangement of highly electron-rich aromatic compounds. Unfortunately, the arrangement of the biphenyl pairs in multiply substituted duplexes is not exactly known. In the absence of a detailed 3-D structure the following discussion is based on the assumption that the zipper-like interstrand intercalation model can also be extended to the triple series.

Obviously there are intrinsically different energetic effects driving the biphenyl–biphenyl and the biphenyl–natural base pair interactions. In order to address the biphenyl–biphenyl interactions separately, we performed a differential thermal stability analysis by subtracting the T_m values of the mono series from those of the triple series. Given that the sequence context and the positioning of the biphenyl units are identical, this seems to be a valid method to identify relative preferences.

The ΔT_m data (Figures 4, 5) clearly show that steric effects do not dominate in the overall association behavior. The thermodynamic parameters of duplex formation (Table 3) indicate that the differences in stability of mono- and triple-substituted dimethoxybiphenyl systems is mostly determined by a favorable change in enthalpy (ΔH), which is not the case in the dinitrobiphenyl systems. There are numerous examples of molecular complex formation in solution characterized by favorable changes in enthalpy, but unfavorable changes in entropy ($\Delta H < 0$; $T\Delta S < 0$).[40] This so called "nonclassical hydrophobic effect" was attributed to favorable changes in solvent cohesive interactions and gains in dispersive interactions. Solvent molecules prefer to interact with bulk solvent molecules rather than to solvate apolar surfaces, and the host and guest molecules tend to interact with each other rather than with solvent molecules. Our calculations do not show any significant differences in polarizability α between the structurally related nitro- and methoxy-substituted biphenyls, thus ruling out a gain in dispersive interactions in the dimethoxy case. Therefore, the nonclassical hydrophobic effect, as observed in the dimethoxy case, is primarily the result of distinct differences in the solvent interactions between dinitro- and dimethoxybiphenyl residues rather than dispersive forces. Alternative effects such as, for example, differences in the structures of the single strands, have also been addressed as a means to explain the different thermochemical behavior. The CD spectra of a dimethoxy- and of a dinitrobiphenyl-carrying single strand (triple series) at different temperatures showed slight differences in ellipticities, especially in the 230–260 nm region (Figure S4, Supplementary Information). In neither case, however, does the temperature series reflect a cooperative structural change, thus ruling out distinctly folded single-strand structures in both systems.

Through integration of all experimental data we currently favor a model in which the self-recognition of the biphenyl residues is mainly solvation-driven. The energetic superiority of the methoxybiphenyl interaction over the nitrobiphenyl interaction seems to be best explained by a nonclassical hydrophobic effect in the former case, based on an energetic gain in ordered solvent–solvent interactions during single strand to duplex transition.

Conclusion

In our previous investigations with unsubstituted biphenyl-*C*-nucleosides we found that up to seven biphenyl residues can be accommodated in the center of a duplex without breakdown of duplex stability.[30,31] We put forward a structural model in which opposing biphenyls recognize each other through zipper-like interstrand intercalation. This model has proven correct for single biphenyl pairs.[32] In the work presented here we show that substitution of the remote phenyl ring of the biphenyl residues with π-donor or -acceptor substituents is possible without the collapse of the double helical structures. In addition, a remarkable indifference of stability as a function of the position of substitution was found. Some modifications lead to increases in stability, exceeding those of natural base pairs. While the biphenyl/natural base pair interactions are energetically dominated

by electrostatic interactions, the biphenyl/biphenyl interactions seem to be dominated by solvation effects.

The flexibility of the biphenyl recognition system as outlined in this work poses at least two relevant questions for further study. Firstly, from the viewpoint of basic supramolecular chemistry it will be of interest to ascertain whether homo- or hetero-biphenyl pairs that are orthogonal to each other in their recognition properties can be found. If such pairs exist, one could imagine the construction of a primitive genetic code based on differences in hydrophobic properties only and not relying on hydrogen bonds for selectivity. Secondly, through incorporation of donor- and acceptor-modified biphenyls with different redox properties into DNA, its charge-transport (electron or hole) properties could be attenuated and expanded beyond the range of that of the four natural bases. This may be interesting for applications in the fields of DNA diagnostics and DNA-based materials.

Experimental Section

Sample Preparation: The syntheses of modified phosphoramidite monomers and their incorporation into oligodeoxynucleotides have been described elsewhere.[33] Unmodified oligodeoxynucleotides were purchased from Microsynth (Balgach, Switzerland) and used without further purification.

Thermal denaturation: All UV melting curves were recorded at least twice at 260 nm on a Cary 3E UV/VIS spectrometer (Varian) fitted with a Peltier block and with the aid of Varian WinUV software. The oligonucleotide concentration was 1.2 μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0. The extinction coefficients for all biphenyl-*C*-nucleosides were experimentally determined and are given in (Table S1 (Supporting Information). Consecutive heating-cooling-heating cycles over the temperature interval of 10 to 90 °C were applied with a linear gradient of 0.5 °C min^{-1}. Heating and cooling ramps were superimposable. Each T_m value (uncertainty ±0.5 °C) was defined as the maximum of the first derivative of the melting curve. For the van't Hoff plots ($1/T_m$ against $\ln c$), T_m values were measured at five different concentrations over the concentration range of 0.5–15 μM (duplex) in the same buffer as described above.

Circular dichroism spectroscopy: Circular dichroism spectra were recorded on a Jasco J-715 spectropolarimeter fitted with a Jasco PFO-350S temperature controller. Subsequently, the graphs were smoothed with a noise filter. The oligonucleotide concentrations were 1.2–3.6 μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0.

Fluorescence spectroscopy: Fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrometer (Varian). The oligonucleotide concentration was 1.2 μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0.

Calculations: Ab initio calculations were performed by use of Gaussian 03 from Gaussian. Restricted Hartree–Fock (RHF) with the basis set 6-31G(d,p) was used for all calculations. log*P* values were calculated with the aid of the program molinspiration (http://www.molinspiration.com).

Acknowledgement

Financial support by the Swiss National Science Foundation (grant-No.: 200020-115913) is gratefully acknowledged.

[1] K. M. Guckian, B. A. Schweitzer, R. X. F. Ren, C. J. Sheils, P. L. Paris, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc.* **1996**, *118*, 8182–8183.
[2] K. M. Guckian, B. A. Schweitzer, R. X. F. Ren, C. J. Sheils, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc.* **2000**, *122*, 2213–2222.
[3] B. A. Schweitzer, E. T. Kool, *J. Am. Chem. Soc.* **1995**, *117*, 1863–1872.
[4] T. J. Matray, E. T. Kool, *J. Am. Chem. Soc.* **1998**, *120*, 6191–6192.
[5] S. Moran, R. X. F. Ren, E. T. Kool, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10506–10511.
[6] K. M. Guckian, J. C. Morales, E. T. Kool, *J. Org. Chem.* **1998**, *63*, 9652–9656.
[7] J. C. Morales, E. T. Kool, *Nat. Struct. Biol.* **1998**, *5*, 950–954.
[8] J. C. Delaney, P. T. Henderson, S. A. Helquist, J. C. Morales, J. M. Essigmann, E. T. Kool, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4469–4473.
[9] T. J. Matray, E. T. Kool, *Nature* **1999**, *399*, 704–708.
[10] K. M. Guckian, T. R. Krugh, E. T. Kool, *Nat. Struct. Biol.* **1998**, *5*, 954–959.
[11] E. T. Kool, J. C. Morales, K. M. Guckian, *Angew. Chem.* **2000**, *112*, 1046–1068; *Angew. Chem. Int. Ed.* **2000**, *39*, 990–1009.
[12] M. Berger, A. K. Ogawa, D. L. McMinn, Y. Q. Wu, P. G. Schultz, F. E. Romesberg, *Angew. Chem.* **2000**, *112*, 3069–3071; *Angew. Chem. Int. Ed.* **2000**, *39*, 2940–2942.
[13] M. Berger, S. D. Luzzi, A. A. Henry, F. E. Romesberg, *J. Am. Chem. Soc.* **2002**, *124*, 1222–1226.
[14] D. L. McMinn, A. K. Ogawa, Y. Wu, J. Liu, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **1999**, *121*, 11585–11586.
[15] A. K. Ogawa, Y. Wu, D. L. McMinn, J. Liu, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **2000**, *122*, 3274–3287.
[16] E. J. L. Tae, Y. Q. Wu, G. Xia, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **2001**, *123*, 7439–7440.
[17] C. Z. Yu, A. A. Henry, F. E. Romesberg, P. G. Schultz, *Angew. Chem.* **2002**, *114*, 3997–4000; *Angew. Chem. Int. Ed.* **2002**, *41*, 3841–3844.
[18] S. Atwell, E. Meggers, G. Spraggon, P. G. Schultz, *J. Am. Chem. Soc.* **2001**, *123*, 12364–12367.
[19] Y. Wu, A. K. Ogawa, M. Berger, D. L. McMinn, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **2000**, *122*, 7621–7632.
[20] S. Matsuda, A. A. Henry, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **2003**, *125*, 6134–6139.
[21] T. Mitsui, A. Kitamura, M. Kimoto, T. To, A. Sato, I. Hirao, S. Yokoyama, *J. Am. Chem. Soc.* **2003**, *125*, 5298–5307.
[22] I. Hirao, M. Kimoto, T. Mitsui, T. Fujiwara, R. Kawai, A. Sato, Y. Harada, S. Yokoyama, *Nat. Methods* **2006**, *3*, 729–735.
[23] N. C. Seeman, *Nature* **2003**, *421*, 427–431.
[24] U. Feldkamp, C. M. Niemeyer, *Angew. Chem.* **2006**, *118*, 1888–1910; *Angew. Chem. Int. Ed.* **2006**, *45*, 1856–1876.
[25] B. Giese, *Acc. Chem. Res.* **2000**, *33*, 631–636.
[26] *Charge Transfer in DNA* (Ed.: H.-A. Wagenknecht), Wiley-VCH, Weinheim, **2005**.
[27] M. A. O'Neill, J. K. Barton, in *Topics in Current Chemistry: Electron Transfer in DNA: I*, Springer, **2007**, pp. 67–115.
[28] K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shionoya, *Science* **2003**, *299*, 1212–1213.
[29] G. H. Clever, C. Kaul, T. Carell, *Angew. Chem.* **2007**, *119*, 6340–6350; *Angew. Chem. Int. Ed.* **2007**, *46*, 6226–6236.
[30] C. Brotschi, C. J. Leumann, *Angew. Chem.* **2003**, *115*, 1694–1697; *Angew. Chem. Int. Ed.* **2003**, *42*, 1655–1658.
[31] C. Brotschi, G. Mathis, C. J. Leumann, *Chem. Eur. J.* **2005**, *11*, 1911–1923.
[32] Z. Johar, A. Zahn, C. J. Leumann, B. Jaun, *Chem. Eur. J.* **2008**, *14*, 1080–1086.
[33] A. Zahn, C. J. Leumann, *Bioorg. Med. Chem.* **2006**, *14*, 6174–6188.
[34] N. S. Hush, A. S. Cheung, *Chem. Phys. Lett.* **1975**, *34*, 11–13.
[35] J. Sponer, J. Leszczynski, P. Hobza, *Biopolymers* **2001**, *61*, 3–31.
[36] S. L. Cockroft, C. A. Hunter, K. R. Lawson, J. Perkins, C. J. Urch, *J. Am. Chem. Soc.* **2005**, *127*, 8594–8595.

[37] F. Cozzi, J. S. Siegel, *Pure Appl. Chem.* **1995**, *67*, 683–689.
[38] S. L. Cockroft, J. Perkins, C. Zonta, H. Adams, S. E. Spey, C. M. R. Low, J. G. Vinter, K. R. Lawson, C. J. Urch, C. A. Hunter, *Org. Biomol. Chem.* **2007**, *5*, 1062–1080.
[39] I. Alkorta, J. J. Perez, *Int. J. Quantum Chem.* **1996**, *57*, 123–135.
[40] E. A. Meyer, R. K. Castellano, F. Diederich, *Angew. Chem.* **2003**, *115*, 1244–1287; *Angew. Chem. Int. Ed.* **2003**, *42*, 1210–1250.

Received: August 28, 2007
Published online: November 26, 2007

